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Perfluoroalkyl substances in aquatic environment-comparison of fish and passive sampling approaches

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ABSTRACT

The concentrations of seven perfluoroalkyl substances (PFASs) were investigated in 36 European chub (*Squalius cephalus*) individuals from six localities in the Czech Republic. Chub muscle and liver tissue were analysed at all sampling sites. In addition, analyses of 16 target PFASs were performed in Polar Organic Chemical Integrative Samplers (POCISs) deployed in the water at the same sampling sites. We evaluated the possibility of using passive samplers as a standardized method for monitoring PFAS contamination in aquatic environments and the mutual relationships between determined concentrations.

Only perfluorooctane sulphonate was above the LOQ in fish muscle samples and 52% of the analysed fish individuals exceeded the Environmental Quality Standard for water biota. Fish muscle concentration is also particularly important for risk assessment of fish consumers. The comparison of fish tissue results with published data showed the similarity of the Czech results with those found in Germany and France.

However, fish liver analysis and the passive sampling approach resulted in different fish exposure scenarios. The total concentration of PFASs in fish liver tissue was strongly correlated with POCIS data, but pollutant patterns differed between these two matrices. The differences could be attributed to the metabolic activity of the living organism. In addition to providing a different view regarding the real PFAS cocktail to which the fish are exposed, POCISs fulfil the Three Rs strategy (replacement, reduction, and refinement) in animal testing.

1. Introduction

Perfluoroalkyl substances (PFASs) are artificially fluorinated hydrocarbons and their derivatives, which have a wide range of industrial, agricultural, and household uses. Due to their surface-active properties, PFASs are used as lubricants, components of fire-fighting foams, and leather and paper surface protectors (Lindstrom et al., 2011; Zhao et al., 2012; Zareitalabad et al., 2013). They are also used in many herbicide and insecticide formulations or in cosmetics (Zhang and Lerner, 2012). PFASs have been produced and used for over sixty years, but due to the lack of suitable analytical methods, they did not arouse scientific interest until early 2000 (Zhao et al., 2012; Valsecchi et al., 2013). Nowadays, PFASs are detected in various environmental matrices all over the world, even in the regions where they have never been used. PFASs are persistent in the environment due to the strong carbon–fluorine bond, which makes them resistant to thermal, chemical, and biological degradation. PFASs are released into the environment from direct (manufacture and application) and indirect sources (release from consumer goods during their use). Aquatic environments are usually the final recipient of these pollutants originating from the industrial areas and waste water treatment plants (WWTPs) of urban centres (Giesy et al., 2001; Prevedouros et al., 2006; Zhao et al., 2012; Zareitalabad et al., 2013).

The most studied PFAS is perfluorooctane sulphonate (PFOS). This compound was reported to be the most prevalent PFAS found in biota samples from various regions of the world (Giesy et al., 2001; Berger et al., 2009; Yeung et al., 2009; Zhao et al., 2012; Naile et al., 2013). Higher PFOS concentrations were found in aquatic mammals and birds that ate fish (Giesy et al., 2001). Significant differences in PFOS concentrations were also found between piscivorous and non-piscivorous fish (Ye et al., 2008b). Besides PFOS, there are some other PFASs, like PFOA, perfluorononanoic acid (PFNA), perfluorohexane sulphonate (PFHxS), or perfluoropentanoic acid (PFPeA), that are usually found in aquatic biota (Giesy et al., 2001; Miege et al., 2012). In contrast to

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other persistent organic pollutants (POPs), which accumulate mainly in the lipid tissues of the body, greater PFAS concentrations are found in the liver, blood plasma, or gall bladder of fish (Giesy and Kannan, 2001; Murakami et al., 2011; Naile et al., 2013). This can be assigned to the indirect exposure pathway, including transformation of precursors such as perfluorooctane sulphonamido ethanols (FOSEs), FOSAs, fluorotelomer alcohols (FTOHs), or polyfluoroalkyl phosphate esters (PAPs) in living organisms (Gebbink et al., 2015).

The toxic effects of some PFASs, including perfluorinated octanesulphonamides (FOSAs), on fish and zooplankton were confirmed by several studies (Sanderson et al., 2004; Zheng et al., 2012). Relationships were found between the vitellogenin gene expression in fish liver, the vitellogenin plasma activity and the PFAS concentrations in fish plasma (Houde et al., 2013). Johansson et al. (2009) found that neonatal exposure to PFOS and perfluorooctanoic acid (PFOA) in mice has negative effects on brain development. For such reasons, PFOS was added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009. Additionally, PFOS was identified as a priority hazardous substance and an Environmental Quality Standard (EQS) of 9.1 $\mu g\ kg^{-1}$ of wet weight (w.w.) for water biota (fish) was set by the Directive of the European Parliament and Council (2013). The European Food Safety Authority (EFSA), (2008) also published a Scientific Opinion on PFOS and PFOA, where the tolerable daily intakes (TDI) of 150 ng kg^{-1} bw d^{-1} and 1.5 $\mu g\ kg^{-1}$ bw d^{-1}, respectively, were set.

Increased knowledge of PFASs characteristics leads to higher demand of its biomonitoring in the environment. Several authors focused on PFASs occurrence in aquatic environment during last decade e.g. (Giesy et al., 2001; Ye et al., 2008a; Berger et al., 2009; Yeung et al., 2009).However, there is no uniform approach for its evaluation in fish; different authors performed analysis in different fish tissues (muscle/muscle with skin/liver/plasma/whole body homogenates) and the units of measurement varied (dry weight/wet weight), which makes it difficult to compare the obtained data and evaluate the real contamination of study areas.

The objectives of this study were to investigate the concentrations of PFAS in water and fish from the Czech Republic and to evaluate differences between monitoring approaches. Polar Organic Chemical Integrative Samplers (POCIS) data were compared with the data from analysis of fish muscle and liver tissue. It seems that POCIS and other passive sampling methods may have the potential to become a standardized approach for biomonitoring of aquatic environments, which fulfils the internationally-established principles of Replacement, Reduction, and Refinement-the Three Rs. These principles are also included in the EU Directive on the protection of animals used for scientific purposes (European Parliament and Council, 2010).

2. Material and methods

2.1. Monitored sites

Six localities belonging to different water courses of the Labe (Elbe) and Morava (Danube) catchments were selected as sampling sites (Fig. 1). These sampling sites were chosen due to higher probability of occurrence of the target pollutants, as they are situated downstream of large cities or industrial areas. There is no evidence about PFAS producers in the Czech Republic, but various kinds of industry could use some of these compounds as ingredients or operating substances in production processes. Communal waste waters can also be an important source of PFASs in rivers downstream urban centres. Detailed information on selected localities, including flow rate or catchment area, is attached in the Supplementary material (Table S1).

Dluhonice lies on the Becva River, downstream of Prerov city

Fig. 1. Map of the Czech Republic with sampling sites.

(47,000 inhabitants), where metal processing, chemical, and photographic industries are located. Valy lies on the Elbe River. This sampling site is situated downstream of Pardubice city (90,000 inh.), where chemical plants, an oil refinery, a heavy machinery factory, and an electronic equipment plant are located. Usti nad Labem lies on the Bilina River, just before its confluence with the Elbe River. The Bilina River is classified as a highly polluted river in the Czech Republic. It flows through the northwestern part of the Czech Republic, which is greatly affected by coal mining activities and has several urban centres, chemical and pharmaceutical industry plants, oil refineries, and metal processing industry plants on its shores. Predmerice is situated on the Jizera River. The Jizera River begins in Poland and flows through mountain areas with several ski resorts (located about 100 km upstream of the sampling site). Two industrial areas are located about 20 and 26 km upstream of Benatky nad Jizerou and Mlada Boleslav, respectively, where the abrasives producers and big automobile producers operate. Nespeky is situated on the Sazava River, where no sources of pollution by PFASs are assumed. Topelec is situated on the Otava River, downstream of Pisek city (30,000 inh.), where several electronic and textile industry producers are located.

2.2. Fish sampling and sample preparation

Six individuals of the European chub (*Squalius cephalus*) were caught at each sampling site in 2012. Chub was chosen as a reference species due to its abundance at all sampling sites and due to its usage as a bio-indicator species in the Czech Hydrometeorological Institute's national programme of surface water quality monitoring.

Experimental animals were handled in accordance with the national and institutional guidelines for the protection of human subjects and animal welfare (European Parliament and Council, 2010). Electrofishing devices were used to obtain a sufficient number of reference fish species at monitored sites. All caught fish were sacrificed then measured, weighed, and scales were taken to determine their age. The characteristics of the sampled fish are given in Table 1. Samples of muscle tissue without skin from the mid-dorsal part of the body were taken, packed into plastic bags, labelled, and stored in insulated boxes on ice during transport to the laboratory; liver samples were taken, packed, labelled, and stored in the same way. For the localities of Predmerice and Valy, only 5 and 3 samples of liver tissue were taken due to the small size of some experimental fish at these sampling sites. All samples were taken individually and then transported to the laboratory, where they were kept frozen at -20 °C until analysis.

2.3. Chemicals and standards

Mixtures of seventeen native (PFAC-MXB) and nine mass-labelled (MPFAC-MXA) perfluorinated acids and perfluoroalkylsulphonates were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Working mixtures of native compounds and surrogate standards were prepared in methanol at 1 μg mL^{-1} and stored at 4 °C. Methanol (LiChrosolv Hypergrade), acetonitrile (LiChrosolv, Hypergrade), toluene (Suprasolv), and dichloromethane (Suprasolv) were purchased from Merck (Darmstadt, Germany). Formic acid, used for acidification of mobile phase, was purchased from Labicom (Olomouc, Czech Republic). Ultrapure water was obtained from an aqua-MAX-Ultra system (Younglin, Kyounggi-do, Korea).

Table 1
Characteristics of caught fish (chub).

Locality	*n*	Age (years)	Body weight (g)	Total body length (mm)
		mean ± SD	mean ± SD	mean ± SD
Nespeky	6	3.2 ± 0.4	140.8 ± 86.8	228.3 ± 35.7
Valy	6	2.3 ± 0.5	67.5 ± 60.7	168.8 ± 46.8
Dluhonice	6	3.7 ± 0.5	255.0 ± 71.6	279.3 ± 26.1
Predmerice	6	3.7 ± 1.1	279.2 ± 352.0	259.8 ± 90.1
Usti nad Labem	6	3.0 ± 0.0	88.3 ± 17.2	207.3 ± 11.4
Topelec	6	3.8 ± 0.9	304.2 ± 150.9	282.2 ± 42.2

2.4. Muscle and liver sample extraction

A modification of the method of (Fedorova et al., 2014) was used for extraction of seven target compounds from fish tissues. Briefly, samples of fish tissue (0.5 g) with added internal standard (20 ng per sample) and 1 mL of extraction solvent (acetonitrile with 1% formic acid) were homogenised at 1800 revolutions per min for 10 min (TissueLyser II, Qiagen, Germany) and centrifuged at 10,000g for 10 min (Micro 200R, Hettich Zentrifugen, Germany). The supernatant was filtered through a syringe filter (0.45 μm pores, regenerated cellulose) and allowed to evaporate to about 0.5 mL at room temperature overnight. An aliquot of the extract was diluted with water (1:1) and this sample was analysed by liquid chromatography with high resolution mass spectrometry (LC-HRMS).

2.5. Passive sampler deployment and extraction

Based on passive sampler calibration results published by (Fedorova et al., 2013), POCIS in the pesticide configuration (triphasic admixture of a hydroxylated polystyrene-divinylbenzene resin, Isolute ENV+) and a carbonaceous adsorbent (Ambersorb 1500) dispersed on a styrene divinylbenzene copolymer (S-X3 Bio Beads) were used (Nya Exposmeter AB, Tavelsjö, Sweden). The samplers were deployed in the riverine water at sampling sites for 21 days during the spring of 2012. After the exposure period, the samplers were retrieved, cleaned with ultrapure water, and transported on ice to the laboratory, where they were stored at −18 °C until analysis. The sampling period of twenty-one days was set to achieve high accumulation of target compounds and representative overview in longer time span.

Sixteen PFASs were analysed in passive samplers after standardized extraction procedures (Alvarez et al., 2005). Briefly, sorbent was transferred into glass gravity-flow chromatography columns filled with glass wool (2 cm layer). Target analytes were recovered from the sorbent by elution with 50 mL of a dichloromethane/methanol/toluene mixture (8:1:1, v/v/v). Extracts were reduced to 2 mL by rotary evaporation. The internal standards (2 ng) were added to the 100 μl sample aliquots in autosampler vials and analysed with LC-HRMS. The complete data set of PFASs in POCIS is reported in the Supplementary materials (Table S2).

2.6. LC-HRMS analysis

A hybrid quadrupole/orbital trap mass spectrometer QExactive (Thermo Fisher Scientific, San Jose, CA, USA), coupled to an Accela 1250 LC pump (Thermo Fisher Scientific, San Jose, CA, USA) and a HTS XT-CTC autosampler (CTC Analytics AG, Zwingen, Switzerland), was used for the analysis. A Cogent Bidentate C18 column (50 mm × 2.1 mm ID × 4 μm particles; Microsolv Technology Corporation, Eatontown, NJ, USA) was used for the separation of target analytes. The gradient and flow of the mobile phase and the basic set-up of the HESI-II ionisation interface are described in the Supplementary materials (Tables S3 and S4, respectively). The mass spectrometer was operated in high resolution product scan

(HRPS) mode to selectively detect the target compounds. The mass width at the isolation quadrupole was set as 0.7 mu. The orbital trap was operated at a resolution of 17,500 FWHM.

Isotope dilution or internal standard method was used in combination with matrix matching standard approach i.e. standard at enough high concentration level was prepared in sample extract.

2.7. Analytical method validation

The method performance for POCIS was already published (Fedorova et al., 2013). Our analytical method for fish tissues was validated regarding its linearity, repeatability, limit of quantification (LOQ), and recovery. The method was linear over the range of 1 to 500 ng g^{-1} (R^2=0.999). Method repeatability was tested for ten replicates; the relative standard deviation (RSD) of replicates was 9%. The recovery of target compounds from fish tissue was studied by spiking "clean" fish samples with a mixture of the target compounds before the extraction procedure. The average recovery of target compounds ranged from 94 to 121% (recovery was tested for 4 concentration levels, 5 replicates for each level). Instrumental LOQ was derived from the calibration curve. Peak area corresponding to this LOQ was used for calculation of LOQs in individual samples with using S/N ratio > 10 as auxiliary parameter in some (mainly liver extract) samples. Corresponding values reflect differences among IS recovery, weight of the samples and final volumes of the extract. LOQs for target compounds in individual samples ranged from 0.27 to 5.4 ng g^{-1} in muscle and from 1.2 to 15.0 ng g^{-1} in liver tissues. All method performance parameters are presented in the Supplementary Materials (Table S5). As internal QA/QC we processed fortified samples with each series of samples. Recoveries of target analytes based on fortified samples were in range from 97% to 123% with exception of PFHxS (152%). Blank samples were prepared during extraction process for both fish tissues and POCIS analyses according to its extraction method. Concentrations of target analytes above the LOQ were not found in blank samples.

2.8. Statistical analysis

A correlation between concentrations of PFAS found in muscle/liver tissues and those found in POCIS was evaluated using Statistica 10 software (StatSoft Inc., USA). As the data did not show a normal distribution, Spearman's correlation was used to quantify the strength of this relation.

3. Results and discussion

The concentrations of target pollutants measured in a set of 72 muscle/liver samples collected in 2012 at 6 sampling sites are presented in Table 2. Similar to other results from the Czech Republic and other geographic regions (Ye et al., 2008b; Rudel et al., 2011; Hradkova et al., 2012; Wang et al., 2012), PFOS was the

Table 2
Concentration of target compounds in samples of fish tissue.

ng g^{-1} (w.w.)	PFPeA		PFHxA		PFHpA		PFHxS		PFOA		PFNA		PFOS		Total analysed PFASs	
	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver
Sampling site Nespeky																
No. of samples	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean	nd	56.0	nd	53.3	nd	14.0	nd	nd	nd	nd	nd	2.3	2.9	10.8	2.9	131.8
SD	nd	27.1	nd	22.3	nd	5.5	nd	nd	nd	nd	nd	1.0	1.5	4.0	1.5	51.6
Minimum	< LOQ	18.0	< LOQ	25.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.0	1.0	7.1	1.0	61.1
Maximum	< LOQ	96.0	< LOQ	91.0	< LOQ	23.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	3.8	5.8	18.0	5.8	206.4
Sampling site Valy																
No. of samples	6	3	6	3	6	3	6	3	6	3	6	3	6	3	6	3
Mean	nd	60.3	nd	54.0	nd	nd	nd	nd	nd	nd	nd	2.8	8.8	64.0	8.8	185.5
SD	nd	30.5	nd	26.5	nd	nd	nd	nd	nd	nd	nd	1.4	1.7	22.6	1.7	86.8
Minimum	< LOQ	30.0	< LOQ	30.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.5	6.3	47.0	6.3	108.5
Maximum	< LOQ	102.0	< LOQ	91.0	< LOQ	13.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	4.8	11.0	96.0	11.0	306.8
Sampling site Dluhonice																
No. of samples	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean	nd	65.3	nd	39.7	nd	nd	nd	nd	nd	nd	nd	1.7	27.3	221.5	27.3	334.0
SD	nd	46.9	nd	19.4	nd	nd	nd	nd	nd	nd	nd	0.6	3.7	86.0	3.7	58.7
Minimum	< LOQ	27.0	< LOQ	21.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.0	22.0	122.0	22.0	230.5
Maximum	< LOQ	161.0	< LOQ	73.0	< LOQ	24.0	< LOQ	< LOQ	< LOQ	11.0	< LOQ	3.0	33.0	381.0	33.0	430.0
Sampling site Predmerice																
No. of samples	6	5	6	5	6	5	6	5	6	5	6	5	6	5	6	5
Mean	nd	81.0	nd	66.8	nd	27.2	nd	nd	nd	nd	nd	2.3	17.1	185.6	17.1	346.5
SD	nd	34.5	nd	38.7	nd	20.9	nd	nd	nd	nd	nd	0.8	6.1	78.4	6.1	130.7
Minimum	< LOQ	26.0	< LOQ	25.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1.6	7.5	86.0	7.5	191.6
Maximum	< LOQ	123.0	< LOQ	138.0	< LOQ	48.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	3.9	26.0	294.0	26.0	531.9
Sampling site Usti nad Labem																
No. of samples	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean	nd	23.0	nd	22.2	nd	5.6	nd	nd	nd	nd	nd	2.0	38.3	466.8	38.3	518.7
SD	nd	5.7	nd	3.9	nd	2.0	nd	nd	nd	nd	nd	1.3	6.1	178.1	6.1	172.2
Minimum	< LOQ	16.0	< LOQ	17.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.7	31.0	319.0	31.0	374.7
Maximum	< LOQ	33.0	< LOQ	29.0	< LOQ	8.4	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	4.6	49.0	804.0	49.0	849.5
Sampling site Topelec																
No. of samples	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean	nd	55.0	nd	21.5	nd	nd	nd	nd	nd	nd	nd	1.2	3.2	18.4	3.2	96.7
SD	nd	34.0	nd	5.6	nd	nd	nd	nd	nd	nd	nd	0.5	1.0	7.9	1.0	35.4
Minimum	< LOQ	13.0	< LOQ	13.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.8	2.2	9.2	2.2	64.4
Maximum	< LOQ	123.0	< LOQ	29.0	< LOQ	3.6	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	2.4	5.3	33.0	5.3	169.8

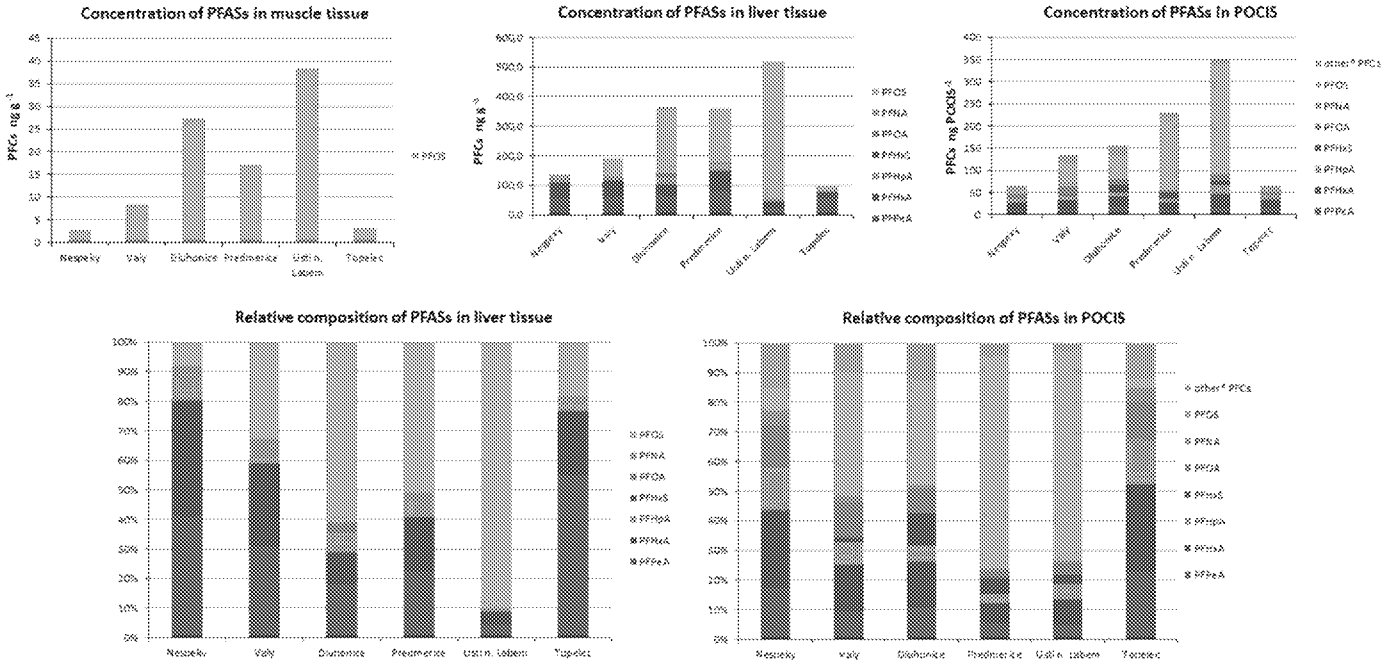

Fig. 2. Concentrations and relative composition of target PFASs in muscle and liver tissue of fish and in POCIS.* 16 PFASs were analysed in POCIS (Supplementary material, Table S2).

prevailing pollutant from a group of monitored PFASs in the aquatic environment.

3.1. Fish muscle tissue

In muscle tissue samples, only PFOS was found above the LOQ. On the other hand, this compound was found in all samples. The arithmetic mean ($n=6$) of PFOS concentrations varied from 2.9 to 38.3 ng g^{-1} w.w., depending on the sampling site. Concentrations exceeding the EQS for aquatic biota were found in 52% of the individual samples. The highest mean and individual (49 ng g^{-1} w.w.) concentrations were found at Usti nad Labem (Bilina River), where the mean concentration of PFOS was more than nine-fold higher than the EQS. The Bílina River is relatively small and, due to low dilution of contaminated inputs, a highly polluted water course, which flows through an area of intensive surface coal mining, heavy chemical industry, and oil refineries.

Few studies focused on monitoring of PFASs in fish from Czech rivers. Unfortunately, some of these works were focused on fish species with different feeding strategies caught at different sampling sites, and the number of samples taken was insufficient to obtain reliable results. Hradkova et al. (2012) found mean concentrations of PFOS that ranged from < LOD to 193 ng g^{-1} w.w. in the muscle tissue of different fish species. The highest mean concentrations in their work were found in the Elbe River, at localities Obristvi (62 ng g^{-1} w.w., $n=3$) and Usti nad Labem (193 ng g^{-1} w.w., $n=1$), in the muscle tissue of bream (*Abramis brama*) and roach (*Rutilus rutilus*), respectively. High concentrations of PFOS were reported by (Hlouskova et al., 2013) from the Bilina River, where the mean concentration of 752 ng g^{-1} w.w. ($n=3$) was detected in the muscle tissue of fish (undefined species). Agreement with our data was found in recent publication, where concentration of PFOS analysed in chub muscle from nine different sampling sites along Elbe and Vltava Rivers ranged between 0.722 and 36.1 ng g^{-1} w.w. (Svihlikova et al., 2015). In any case, PFOS seems to be a relevant environmental pollutant in the Czech aquatic environment.

Similar investigations were made in France at several sampling sites along the Rhone River. Samples of muscle tissue with the skin of several fish species were analysed and the concentrations of PFOS ranged from 15.7 to 308.9 ng g^{-1} d.w. (Miege et al., 2012). The occurrence of PFASs in fish from Lake Vättern (Sweden) was reported by (Berger et al., 2009), who observed median concentrations of PFOS in the muscle tissue of different fish species from this locality that varied between 2.86 ng g^{-1} w.w. (*Coregonus lavaretus*) and 12 ng g^{-1} w.w. (*Lota lota*). (Ye et al., 2008b) examined the Mississippi River and analysed the muscle tissue of carp (*Cyprinus carpio, n 30*) at three different sampling sites; concentrations of PFOS ranged from 4.3 to 90 ng g^{-1} w.w. As occurred in our study, PFOS was the dominant compound at each of the three monitored localities of the Mississippi River, comprising 77% to 89% of the total amount of analysed PFASs in muscle tissue. Lower concentrations of Perfluoroalkyl acids (PFAAs) than in freshwater ecosystems are reported in fish from Baltic Sea (Koponen et al., 2015). Concentrations of PFOS in muscle samples of various fish species caught in the open sea sampling sites varied from 0.31 to 7.5 ng g^{-1} w.w. Similar concentrations (from < LOD to 5.66 ng g^{-1} w.w.) were found in fish from 9 sampling sites within Aegean Sea (Greece, Turkey). Ten sites were investigated in total during this study and different fish species was caught at each site. Different from all others was Picarel (*Spicara smaris*) as 20.4 ng g^{-1} w.w. of PFOS was analysed in its muscle (Vassiliadou et al., 2015). Because of the design of experiment it is impossible to say, if this difference is species or site specific.

3.2. Fish liver tissue

In contrast to samples of muscle tissue, concentrations of four analytes, PFOS, PFNA, PFPeA, and PFHxA, were above the LOQ in 100% of individual liver samples (Table 2). PFHpA was above the LOQ in 44% of individual liver samples. Concentrations of PFHxS and PFOA were below their LOQs in all individual liver samples with only one exception in case of PFOA at locality Dluhonice. PFASs pattern and higher concentrations of target analytes correspond with other reports that analysed PFASs in the liver samples of fish, and this tissue is recognised as a main organ of

bioaccumulation of these extraneous substances in the fish body (Giesy and Kannan, 2001; Prevedouros et al., 2006; Rudel et al., 2011).

PFOS was dominant in liver samples from sampling sites Valy, Dluhonice, Predmerice, and Usti nad Labem, where it reached 35%, 66%, 55%, and 90%, respectively, of total PFAS concentrations (Fig. 2).

In sampling sites Nespeky and Topelec, the contribution of PFOS to the total PFAS concentration was only 8% and 19%, respectively. PFPeA was the dominant compound from a group of analysed PFASs in these two localities, with contributions of 43% and 57%, respectively. Locality Topelec lies on the Otava River downstream the city of Pisek (population of 30,000), where several electronic and textile industry producers are located, while locality Nespeky lies on the Sazava River in a rural area, where no sources of pollution by PFASs are known.

The Bilina River (sampling site Usti nad Labem) was the most contaminated among the monitored localities. As it was mentioned before, the Bilina River is one of the most polluted water courses in the Czech Republic, in general. A total PFAS concentration of 519 ng g^{-1} w.w. was registered there (arithmetic mean, $n=6$), 90% of which was PFOS. Several authors, focusing on the occurrence and fate of PFASs, mentioned that PFOS and PFOA can be formed as breakdown products of other PFASs (Takagi et al., 2008; Kaserzon et al., 2012; Rahman et al., 2014). Based on these findings, we assume that there are many local sources of pollution in the catchment area of the Bilina River. As the sampling site is located near the confluence with the Elbe River, it is possible that some of these parental PFASs are transformed into PFOS, leading to its great contribution on total PFAS concentration there. On the other hand, PFOA, which also can be formed by breakdown of certain precursor compounds, was not found in fish tissues.

Rudel et al. (2011) analysed the liver samples of bream that originated from several water courses (Elbe, Rhine, and Saar Rivers) and one reference background site (Lake Belau) in Germany. These samples were retrieved from the German ESB archive and correspond to the years 2007 and 2008. The concentrations of PFOS in liver samples of bream from the rivers ranged from 130 to 257 ng g^{-1} w.w.; it was only 6.4 ng g^{-1} w.w at the reference site. In our study, concentrations of PFOS in samples of chub liver ranged from 10.8 to 466 ng g^{-1} w.w. (arithmetic mean, $n=6$). This observation fits surprisingly well to Rudel's finding, as the lowest PFOS level Czech site is considered a "clean" area and high ones correspond to similarly industrialised areas in Germany.

3.3. POCIS

Unlike fish tissues, seven target analytes were found in POCIS at levels above the LOQ at all monitored sites, with two exceptions concerning PFHxS at sampling sites Topelec and Nespeky (Table 3). At most of the monitored sites, PFOS was the dominant compound among the analysed PFASs, with concentrations from 2.6 to 250 ng $POCIS^{-1}$. Two exceptions were found at Topelec and Nespeky again, where PFOS reached only 5% and 10%, respectively, of the total PFAS concentrations. PFPeA and PFHxA were the predominant compounds in POCIS extracts from these sampling sites. The results from POCIS analyses correspond to those from fish liver, where the Topelec and Nespeky sites also significantly differed from others. Besides the seven target analytes, another nine were analysed in POCIS. The concentrations of these nine compounds varied from < LOQ to 8.3 ng $POCIS^{-1}$ per POCIS and they were only a minor component of PFAS pollution; see Supplemetary material (Table S2).

3.4. Comparison of studied approaches

The total PFAS concentrations found in the POCIS strongly correlate with those obtained from the liver tissue of fish ($r=0.94$, $p<0.05$). From target analytes, only PFOS was found in muscle tissue of fish and its concentrations also positively correlated between fish and POCIS ($r=0.84$, $p<0.05$).

The comparison of data obtained from analysis of different fish tissues and from POCIS at all monitored sites are presented in Fig. 2. From the relative composition of target compounds in liver tissue and POCIS, it is obvious that we can obtain quite different pictures about PFAS contamination patterns. This fact is supported by the lack of significant correlation between concentrations of individual PFASs found in liver tissue and in POCIS at sampling sites. These differences could be caused by fish metabolic activity, which can transform some of the PFASs into others (Takagi et al., 2008; Rahman et al., 2014) or to fast excretion of some compounds.

Concerning adult fish data, its reliability could be affected by fish size and age too. Different view on real PFASs cocktail could be also made because of metabolic activity of live fish. Besides its relevance and ecological/ethical consequences, the economy and time costs of these two approaches differ significantly.

As the fish liver was recognised as a relevant tissue for PFAS monitoring (Giesy et al., 2001; Nalle et al., 2013), passive sampling seems to be an even better alternative to live animals for monitoring these compounds in aquatic environments. This approach avoids the sacrifice of aquatic biota (mostly fish), so it fulfils the Three Rs (Replacement, Reduction, and Refinement). These principles are also included in the EU Directive on the protection of animals used for scientific purposes (European Parliament and Council, 2010).

Table 3
Concentration of target compounds (ng $POCIS^{-1}$) in passive samplers at monitored sampling sites.

Sampling site	t (°C)[a]	PFPeA ng $POCIS^{-1}$	PFHxA ng $POCIS^{-1}$	PFHpA ng $POCIS^{-1}$	PFHxS ng $POCIS^{-1}$	PFOA ng $POCIS^{-1}$	PFNA ng $POCIS^{-1}$	PFOS ng $POCIS^{-1}$	other PFASs[b] ng $POCIS^{-1}$	Total PFASs ng $POCIS^{-1}$
Nespeky	17	11	17	9.1	0	9.1	3	5.3	9.5	64
Valy	19	13	21	9.9	2.1	16	2.9	56	13.1	134
Dluhonice	19	17	24	8.3	17	13	2	56	18.7	156
Predmerice	16	13	15	7.3	13	5.4	1.3	165	10	230
Usti n. Labem	17	16	31	19	10	14	2.7	250	7.3	350
Topelec	[c]	15	19	9.7	0	8.4	2.9	2.6	7.4	65

[a] Mean water temperature counted from daily measured values.
[b] Sixteen different PFASs were analysed in POCIS, for more information see Supplementary material (Table S2).
[c] No data because of technical problem.

4. Conclusion

As other authors have indicated, there is a high variability in the levels of PFAS contamination of aquatic environments. The level of contamination is affected by the occurrence of large urban centres and various types of industrial production. Fish livers were better indicators of PFAS pollution than muscle because five of the seven target compounds were found above the LOQ in liver, while only PFOS was found above the LOQ in muscle.

Passive sampling (POCIS) seems to be a more effective approach for monitoring of PFASs in aquatic environments than analyses of fish. Although the total concentrations of PFASs found in POCIS strongly correlated to those found in the liver tissue of fish, the composition of the individually measured substances and their ratios in POCIS and fish liver were different. This difference is probably caused by metabolic transformation of some PFASs in fish or their excretion. There are also other reasons to eliminate the use of fish for this type of monitoring, such as expenses and ecological and ethical aspects. On the other hand, the muscle tissue of fish remains an important indicator for risk assessment of consumers.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2015.11.010.

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